Date Pits Alleviate Reproductive Disorders in Male Diabetic Rats

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Abstract: The present study aimed to investigate the role of date pits powder (DP) in restoring reproductive functions in male diabetic rats. Chemical composition of DP was determined. Forty male rats weighing (150-170 g) were divided into five groups (n=8 rats) as follows: group 1 (control) fed basal diet, group 2 (diabetic rats) fed basal diet, groups 3, 4 and 5 (DP-treated diabetic rats) fed basal diets supplemented with DP at three levels (5, 10 and 15 %). The diet formula in DP-treated diabetic groups was adjusted for their nutritive values. Results showed that date pits contain high percent of fiber (21.54%), while fat and protein amounted 8.17% and 5.47% (dry weight basis), respectively. Diabetic group showed significant reduction in body weight gain, remarkable change in sperm characteristics as evidenced by significant decline in sperm count, motility and viability accompanied by significant elevation of sperm abnormalities. Moreover, significant decrease in serum testosterone level and significant increase in serum glucose level have been recorded to diabetic group to that in control group. Diabetes also induced enhancement in oxidative stress in rats as indicated by significant elevation in thiobarbituric acid reactive substance formation (TBARS) level and the reduction in superoxide dismutase (SOD) activity in testicular tissues. Additionally, significant hyperlipidemia has been demonstrated in diabetic rats with respect to in control. Counterparts on the other side, supplementation of DP in the diet of diabetic rats induced significant increase in body weights, remarkable improvement in sperm characteristics and glycemic state, increase in serum testosterone level, decrease in TBARS and increase in SOD activity in testicular tissue and improvement in lipid profile, which they almost resettled near the control level. The alleviated effect of DP was concentration dependent. Examination of testis tissues in diabetic group revealed testicular degeneration with absence of spermatogonial cells, as well as necrosis of germ cells and interstitial oedema. Feeding DP overcomes the remarkable changes in the structural organization of testicular tissues; especially the high concentration (15 %) showed hyperactivation of germ cells with impaction of seminiferous lumen with sperm. Therefore, this study demonstrates that DP possesses hypoglycemic, antioxidant and hypolipidemic activities as well as it has promising effect against diabetic induced reproductive disorders.

Key words: Date pits • Antioxidant • Diabetes • Reproductive disorders • Rats

INTRODUCTION

Diabetes mellitus (DM) is a chronic progressive metabolic disorder characterized by hyperglycemia that causes severe complications and deleterious effects on various organs in the body. There are growing evidences proving that the excess generation of reactive oxygen species (ROS) in diabetes, which cause oxidative stress, may wholly or in part contribute towards the development of complications in a variety of tissues [1, 2].

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Currently, reproductive dysfunctions in diabetic males are well documented, as alarming high rate of infertility has been reported in diabetic males [3, 4]. Diabetes was found to adversely affect the sperm as it has been reported that, diabetic men have high percentage of sperm nuclear DNA fragmentation and apoptosis [5]. A recent study of Bhattacharya et al. [6] reported that husbands of infertile couples with diabetes possess lower volume of ejaculates, sperm count and percentage of motile sperm as compared to healthy husbands of couples with proven fertility. Similarly, Thakur et al. [7] reported that hyperglycemia in rats diminished sperm count, seminal fluid fructose and antioxidant enzymes.

The diabetogenic agent Streptozotocin (STZ), isolated from Streptomyces achromogenes is a genotoxic agent and a potential source of oxidative stress. The STZ-induced diabetes rat model is widely used in basic studies to evaluate the effects of DM on male infertility [8]. Shrilatha and Muralidhara [9] have demonstrated the increased oxidative stress in the STZ-induced diabetic model which greatly participates in the development of spermatogenic dysfunction. The seed of the date palm tree (Phoenix dactylifera L.), which constitutes 10-15% of the fruit weight is a byproduct of date processing industries [10]. With world production of dates reaching 9 million tons ~ 960 thousand tons of seeds are produced [11] and being wasted or partly used as fodder, noncaffeinated coffee, or a source of dietary fibers [12]. It has been shown that it possesses excellent nutritional qualities and represents a good source of bioactive components [13, 14]. The determination of the macro-and micro-nutrient profiles of eighteen varieties of date seeds from date fruits cultivated in the UAE showed that date seeds contain high amounts of fiber (67.6 -74.2 g/100 g) and considerable amounts of some minerals, vitamins, lipids and protein [13, 15]. Additionally, date seeds were shown to be rich in antioxidants, for which antihyperlipidemic, anticancer and antimutagenic properties were identified. The determination of their polyphenolic profile by UPLC-DAD-ESI-MS, Habib et al. [14] revealed a total amount of polyphenols of 50.2 mg/g, with the primary compounds being epicatechin and catechin, whose antioxidant properties are well established [16]. Date seeds have also been shown to exert in vitro and in vivo antioxidant effects [17]. Keeping the balance between the production of ROS and their catabolism by antioxidants is a critical mechanism in preventing damage from oxidative stress. Therefore, supplementation with antioxidants has been used to prevent STZ-induced reproductive dysfunctions [18]. Although date seeds are readily available, few studies have been undertaken to prove their antidiabetic [19] and antioxidative activity [17]. In this study we aimed to elucidate the possible role of date pits powder in restraining the reproductive functions in male. This goal could be achieved through investigating the antidiabetic, antioxidant, hypolipidemic effect as well as their effect on testicular functions and structure in STZ-induced diabetic rats.

**MATERIALS AND METHODS**

Date pits (as a waste) was purchased from Jam Factory, pilot plant, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt. The date pits were oven dried at 30-40°C, ground to fine powder with a heavy-duty grinder and kept in a refrigerator (4°C) till use. Male albino rats were purchased from the Animal House Colony of the National Research Centre, Dokki, Egypt. Streptozotocin (STZ) and chemicals with highest laboratory purity were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Kits were purchased from Sigma-Aldrich Co. USA. Casein, sucrose and cellulose were purchased from Morgan Company, Cairo, Egypt. Corn oil and corn starch obtained from local market, Cairo, Egypt.

**Chemical Analysis of Date Pits:** Date pits powder (DP) was chemically analyzed to determine the following: moisture, crude protein, crude fat, crude fiber and ash contents according to A.O.A.C. [20], while total carbohydrates content was calculated by difference.

**Experimental Animals:** A total number of 40 adult male albino rats weighing 150 -170 g were used in this study. They were kept in the animal house for one week for proper acclimatization before starting the experiment under the same controlled laboratory conditions of illumination (12 h light/12 h darkness), temperature 20-25°C and ventilation. They were housed in stainless steel cages, maintained on standard rodent diet [21] and water ad libitum throughout the experimental period.

**Induction of Diabetes:** Induction of diabetes was carried out following the method described by Maiti et al. [22]. Rats were fasted for 12 hrs before STZ injection. Freshly prepared STZ solution was injected in rats intraperitoneally (i.p.) as a single dose (50 mg/kg b.wt in 0.2 ml of 0.05 M. citrate buffer, pH: 4.5), then animals were allowed to drink 5% glucose solution overnight to
minimize death from hypoglycemia [23]. Seventy-two hrs later, blood samples were obtained from retro-orbital venous plexus of each rat by a fine capillary glass tube and the blood glucose concentration was determined to confirm induction of diabetes, the non-diabetic rats were excluded from the study, animals with blood glucose levels > 300 mg/dl were considered diabetic and used in this experiment.

Experimental Design: The experimental animals were divided into five groups, each of eight rats as follows:

*Group 1:* Negative control rats fed basal diet.
*Group 2:* Diabetic untreated rats fed basal diet.
*Group 3, 4 and 5:* Diabetic rats fed basal diets supplemented with date pits powder (DP) at concentrations of 5, 10 and 15 %. The diet formula in DP-treated diabetic groups was adjusted for the nutritive values. The duration of the experimental lasted for 70 days for competition of the spermatogenic cycle and maturation of sperms in epididymis [24]. Animals were weighted twice weekly. At the end of the experimental period, rats were fasted overnight before blood samples collection and scarification. The collected blood samples were centrifuged to separate serum then stored at -20°C until biochemical analysis.

Semen Characteristics: Seminal content of epididymis was obtained by cutting of cauda epididymis using surgical blades and squeezed in a sterile clean watch glass. This content was diluted 10 times with 2.9 % sodium citrate dehydrate solution and thoroughly mixed to estimate the progressive motility and sperm count [25]. One drop of the suspension was smeared on a glass slide and stained by Eosin-nigrosin stain to determine the percentage of sperm cell viability and morphological abnormalities [26]. Abnormal head and tails were evaluated according to Mori et al. [27] and Okamura et al. [28].

Biochemical Analysis: Glucose level was estimated based on glucose oxidize determined method [29]. Serum testosterone level was estimated using method of Ismail [30]. Serum total cholesterol (TC) [31], triglyceride (TG) [32] and high density lipoproteins cholesterol (HDL-C) [33], while low density lipoproteins cholesterol (LDL-C) and very low density lipoproteins cholesterol (VLDL-C) concentration were calculated according to Friedwald et al. [34].

Determination of Testicular Antioxidant Enzymes: After scarification, one testes of each rat was used for estimation of antioxidant enzymes and lipid peroxidation. One gram of testicular tissue was weighed after ice water washing and homogenized in 9 volume buffered saline 0.9 %, centrifuged at 4000 rpm at 4°C for 15 min., the supernatant was collected and kept at -20°C till further investigation. Testicular thiobarbituric acid reactive substance formation (TBARS) was determined according to the method of Esterbauer and Cheeseman [35] and superoxide dismutase activity (SOD) was determined according to Giannopolitis and Ries [36].

Histopathological Examination: The other testes from each rat in the different studied groups was washed with the normal saline solution to remove blood, fixed in 10% neutral formalin for a period of at least 24 hrs, dehydrated in different grades of alcohol and processed for paraffin embedding. Sections of 5 μm thickness were cut using a rotary microtome. The sections were processed and passed through graded alcohol series, stained with Haematoxylin and Eosin, cleared in xylene and examined microscopically [37].

Statistical Analysis: Results were expressed as a mean ± SD. Data were analyzed statistically by analysis of variance, for statistical significance (P ≤ 0.05) using one way ANOVA, post hoc multiple comparisons [38].

RESULTS

Date Pits Chemical Composition: Results for chemical composition of date pits are shown in Table 1. The moisture, protein, fat, ash and fiber contents (dry weight basis) were 5.17, 5.47, 8.17, 0.86 and 21.54 %, respectively. Accordingly, total carbohydrate content was 58.79 %.

Body Weight: As shown in Table 2, there were very highly significant decrease in final body weight and BWG% in diabetic group as compared with the control group (p<0.001). Diabetic groups treated with diets supplemented with different concentrations of DP showed very highly significant difference compared with diabetic untreated group at the three concentration levels. At the same time there was significant difference between group treated with diet supplemented with 5% DP and the other two treated groups (10 &15 %) in finial body weight and BWG%. The improvement in body weight was concentration dependent. There was insignificant
Table 1: Chemical composition of date pits / 100 g powder

<table>
<thead>
<tr>
<th></th>
<th>DP/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (g)</td>
<td>5.17± 0.25</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>5.47 ± 0.21</td>
</tr>
<tr>
<td>Total fat (g)</td>
<td>8.17 ± 0.27</td>
</tr>
<tr>
<td>Ash (g)</td>
<td>0.86 ± 0.05</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>21.54± 0.28</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>58.79± 0.63</td>
</tr>
</tbody>
</table>

DP: Date Pits
Each value is the mean of 3 determinations ± SD.
All values on dry weight basis.

Table 2: Body weight gain and percent in control, diabetic and DP-treated rats

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>BWG %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-ve)</td>
<td>162.88 ± 5.59</td>
<td>271.40 ± 6.53</td>
<td>66.63 ± 6.38</td>
</tr>
<tr>
<td>DM</td>
<td>161.25 ± 6.27</td>
<td>206.03 ± 6.20</td>
<td>27.77 ± 4.09***</td>
</tr>
<tr>
<td>DM+ DP 5%</td>
<td>162.5 ± 6.96</td>
<td>241.35 ± 7.51*</td>
<td>48.75 ± 3.82***</td>
</tr>
<tr>
<td>DM+ DP 10%</td>
<td>163.75± 5.78</td>
<td>258.50 ± 12.85*</td>
<td>57.86 ± 5.37***</td>
</tr>
<tr>
<td>DM+ DP 15%</td>
<td>160.88± 6.89</td>
<td>260.77 ± 12.52*</td>
<td>62.09 ± 4.41***</td>
</tr>
</tbody>
</table>

DP: Date Pits, DM: Diabetes, BWG %: Body weight gain percent.
- Values are mean ± SD (n=8/ group).
  * Significant difference from control group.
  + Significant difference between DM group and diabetic groups treated with DP.
  ** Significant difference between diabetic group treated with DP 5% and DM groups treated with DP 10 &15 %.
  (p < 0.05, *p < 0.01 and ** p < 0.001).

Table 3: Sperm characteristics in control, diabetic and DP-treated rats

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Count (10⁶ ml)</th>
<th>Motility (%)</th>
<th>Viability (%)</th>
<th>Sperm abnormalities (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-ve)</td>
<td>66.32 ± 5.94</td>
<td>85.44 ± 7.37</td>
<td>91.09 ± 8.51</td>
<td>7.23 ±0.58</td>
</tr>
<tr>
<td>DM</td>
<td>40.00 ± 3.25***</td>
<td>51.25± 4.49***</td>
<td>60.09 ± 6.05***</td>
<td>17.18±1.54***</td>
</tr>
<tr>
<td>DM+ DP 5%</td>
<td>52.25 ± 5.16***</td>
<td>64.00± 3.42***</td>
<td>74.38±7.17***</td>
<td>13.42 ± 1.24***</td>
</tr>
<tr>
<td>DM+ DP 10%</td>
<td>59.13±5.11***</td>
<td>74.38±7.17***</td>
<td>82.63±4.69***</td>
<td>8.75 ±0.86***</td>
</tr>
<tr>
<td>DM+ DP 15%</td>
<td>64.63± 6.02***</td>
<td>81.38±7.44***</td>
<td>88.13±6.66***</td>
<td>7.7 ±0.76***</td>
</tr>
</tbody>
</table>

DP: Date Pits, DM: Diabetic.
-Values are mean ± SD (n=8/ group).
  * Significant difference from control group.
  + Significant difference between DM group and diabetic groups treated with DP.
  ** Significant difference between diabetic group treated with DP 5% and DM groups treated with DP 10 &15 %.
  (p < 0.05, * p < 0.01 and ** p < 0.001).

Table 4: Glucose and testosterone levels in control, diabetic and DP-treated rats

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Glucose (mg/dl)</th>
<th>Testosterone (ng/ ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-ve)</td>
<td>106.16 ± 10.19</td>
<td>1.75±0.14</td>
</tr>
<tr>
<td>DM</td>
<td>250.59 ± 10.96***</td>
<td>0.82 ±0.06***</td>
</tr>
<tr>
<td>DM+ DP 5%</td>
<td>176.74 ± 11.03***</td>
<td>1.09±0.08***</td>
</tr>
<tr>
<td>DM+ DP 10%</td>
<td>130.78 ± 9.01***</td>
<td>1.49±0.13***</td>
</tr>
<tr>
<td>DM+ DP 15%</td>
<td>121.14 ± 11.58***</td>
<td>1.61±0.15***</td>
</tr>
</tbody>
</table>

DP: Date Pits, DM: Diabetic.
-Values are mean ± SD (n=8/ group).
  * Significant difference from control group.
  + Significant difference between DM group and diabetic groups treated with DP.
  ** Significant difference between diabetic group treated with DP 5% and DM groups treated with DP 10 &15 %.
  (p < 0.05, * p < 0.01 and ** p < 0.001)
difference \( (p > 0.05) \) between rats group treated with diet supplemented with 10 % DP and those treated with diet supplemented with 15 % DP in final body weight and BWG%.

**Sperm Characteristics:** Data presented in Table 3 showed the effect of DP on sperm characteristics in diabetic rats the data revealed that, diabetic group showed very highly significant reduction in terms of sperm count, sperm motility and sperm viability rates, but had very highly significant elevation in abnormal sperm (head and tail) rates \( (p < 0.001) \) as compared with the control group. Supplementation of DP in the diets of diabetic treated groups induced marked improvement in sperm characteristics in a concentration dependent manner; there were very highly significant \( (p < 0.001) \) amelioration as compared with diabetic untreated group. The most improvement was recorded in diabetic group treated with diet supplemented with DP at concentration 15 %.

**Glucose and Testosterone Levels:** Glucose levels in control, diabetic rats and diabetic rats treated with different concentration of DP in the diet are presented in Table 4. Significant hyperglycemia \( (p < 0.001) \) was found in diabetic untreated group as compared with the control group. Treatment with DP at the three concentration levels induced very highly significant decrease in blood glucose level \( (p < 0.001) \) as compared with diabetic untreated group. At the same time, there was very highly significant difference \( (p < 0.001) \) between diabetic treated with 5% DP supplemented diets and the other two groups (10 &15%), with insignificant \( (p > 0.05) \) change between 10 % and 15 % concentration of DP-treated groups. Concerning testosterone level, diabetes induced marked decrease in testosterone level; there was very highly significant reduction \( (p < 0.001) \) compared with the control group. Diabetic rats treated with DP supplemented diet showed significant improvement in serum testosterone level in a dose dependent manner; there was very highly significant increase \( (p < 0.001) \) as compared with diabetic untreated group. Diabetic group treated with 5 % DP supplemented diet showed very highly significant difference \( (p < 0.001) \) as compared with both diabetic treated with 10 % and 15% supplemented diets, while there was insignificant difference \( (p < 0.05) \) between 10% and 15 % concentration DP- treated groups.

Table 5: Testicular antioxidant enzymes in control, diabetic and DP- treated rats

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>TBARS (n mol/mg protein)</th>
<th>SOD (u/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-ve)</td>
<td>0.097 ± 0.013</td>
<td>0.089 ± 0.009</td>
</tr>
<tr>
<td>DM</td>
<td>0.201 ± 0.02***</td>
<td>0.039 ± 0.016***</td>
</tr>
<tr>
<td>DM+ DP 5%</td>
<td>0.173 ± 0.016***</td>
<td>0.058 ± 0.014***</td>
</tr>
<tr>
<td>DM+ DP 10%</td>
<td>0.120 ± 0.01***</td>
<td>0.072 ± 0.012***</td>
</tr>
<tr>
<td>DM+ DP 15%</td>
<td>0.103 ± 0.01***</td>
<td>0.086 ± 0.009***</td>
</tr>
</tbody>
</table>

DP: Date Pits, DM: Diabetic, TBARS: Thiobarbituric acid reactive substance, SOD: Superoxide dismutase.

- Values are mean ± SD (n=8/ group).
- \(^a\) Significant difference from control group.
- \(^b\) Significant difference between DM group and diabetic groups treated with DP.
- \(^c\) Significant difference between diabetic group treated with DP 5% and DM groups treated with DP 10 &15 %.
- \(^d\) Significant difference between diabetic group treated with DP 10% and DM group treated with DP 15 %.

\((p < 0.05, \quad ^* p < 0.01 \quad \text{and} \quad ^{**} p < 0.001)\).

Table 6: Total cholesterol, triglycerides and high density lipoprotein-cholesterol levels in control, diabetic and DP-treated rats

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-ve)</td>
<td>89.44 ± 6.08</td>
<td>82.46 ± 6.98</td>
<td>62.86 ± 4.99</td>
</tr>
<tr>
<td>DM (+ve)</td>
<td>195.37 ± 18.61***</td>
<td>151.68 ± 7.79***</td>
<td>30.79 ± 2.24***</td>
</tr>
<tr>
<td>DM+ DP 5%</td>
<td>142.51 ± 13.71***</td>
<td>130.77 ± 8.25***</td>
<td>45.59 ± 3.51***</td>
</tr>
<tr>
<td>DM+ DP 10%</td>
<td>108.08 ± 10.43***</td>
<td>95.32 ± 8.13***</td>
<td>57.35 ± 4.06***</td>
</tr>
<tr>
<td>DM+ DP 15%</td>
<td>97.61 ± 9.22***</td>
<td>89.41 ± 7.87***</td>
<td>61.13 ± 4.02***</td>
</tr>
</tbody>
</table>

DP: Date Pits, DM: Diabetic, TC: Total cholesterol, TG: Triglyceride, HDL-C: High density lipoproteins cholesterol.

- Values are mean ± SD (n=8/ group).
- \(^a\) Significant difference from control group.
- \(^b\) Significant difference between DM group and diabetic groups treated with DP.
- \(^c\) Significant difference between diabetic group treated with DP 5% and DM groups treated with DP 10 &15 %.

\((p < 0.05, \quad ^* p < 0.01 \quad \text{and} \quad ^{**} p < 0.001)\).
Table 7: Low density lipoprotein-cholesterol and very low density lipoprotein-cholesterol levels in control, diabetic and DP-treated rats.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>LDL-C (mg/dl)</th>
<th>VLDL-C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-ve)</td>
<td>10.09 ± 0.92</td>
<td>16.49 ± 1.45</td>
</tr>
<tr>
<td>DM</td>
<td>134.24± 12.02***</td>
<td>30.34 ± 1.90****</td>
</tr>
<tr>
<td>DM+DP 5%</td>
<td>70.77 ± 6.53****</td>
<td>26.15 ± 0.76***</td>
</tr>
<tr>
<td>DM+DP 10%</td>
<td>31.67 ± 2.82****</td>
<td>19.06 ± 1.35***</td>
</tr>
<tr>
<td>DM+DP 15%</td>
<td>18.6 ± 1.70****</td>
<td>17.88 ± 1.84***</td>
</tr>
</tbody>
</table>

DP: Date Pits, DM: Diabetic, LDL-C: Low density lipoproteins cholesterol, VLDL-C: Very low density lipoproteins cholesterol.
- Values are mean ± SD (n=8/ group).
- Significant difference from control group.
- Significant difference between DM group and diabetic groups treated with DP.
- Significant difference between diabetic group treated with DP 5% and DM groups treated with DP 10 &15 %.
- Significant difference between diabetic group treated with DP 10% and DM group treated with DP 15 %.

Testicular Oxidant/Antioxidant Status: Results indicated that, diabetes caused highly significant elevation in TBARS level accompanied with very highly significant decline in SOD activity compared with the control group. Supplementation of DP in the diets of diabetic groups produced significant improvement in oxidant/antioxidant status in a concentration dependent manner. There was very highly significant difference as compared with diabetic untreated group. There was very highly significant difference between diabetic group treated with 5 % DP supplemented diet and the other two treated groups (10 &15 %). At the same time there was significant (p <0.05) change between 10 &15% DP- supplemented groups in both TBARS and SOD (Table 5).

Lipid Profile Parameters: The effect of feeding DP on lipid profile in diabetic rats is illustrated in Tables 6 and 7. The results revealed that, there was very highly significant (p<0.001) increase in TG, TC, VLDL-C and LDL-C combined with very highly significant (p< 0.001) decrease in HDL-C in diabetic untreated group as compared with control (-ve) group. Feeding diabetic groups diets supplemented with DP improved these parameters. There was very highly significant difference (p<0.001) as compared with diabetic untreated group in a concentration dependent manner in all tested lipid parameters. At the same time the data showed that, diabetic rats treated with 5 % DP supplemented diet showed very highly significant difference (p<0.001) as compared with diabetic treated with 10 and 15 % supplemented diets, while insignificant difference (p<0.05) was seen between 10% and 15% concentration levels of DP except in LDL-C value.

Histopathological Examination: Microscopical examination of testis in the control rats showed normal histopathological structure of seminiferous tubules (Fig. 1). Meanwhile, testis of diabetic rats showed marked testicular degeneration with complete absence of spermatogonial cells (germ cells) (Figs 2 and 3), necrosis of germ cells and interstitial oedema (Fig. 4). Testis of diabetic rats treated with 5 % DP showed congestion of interstitial blood vessels and slight degeneration of spermatogonial cells lining seminiferous tubules (Figs. 5 and 6). Testis of diabetic rats treated with 10 % DP showed apparent normal seminiferous tubules, expect some congestion of interstitial blood vessels (Figs. 7 and 8), while testis of diabetic rats treated with 15 % DP showed hyper-activation of germ cells with impaction of seminiferous lumen with sperms (Figs. 9 and 10).
Fig. 3: Testis of diabetic rats showing degeneration of spermatogonial cells lining seminiferous tubules. (H& Ex 400)

Fig. 4: Testis of diabetic rats showing marked testicular degeneration and necrosis of germ cells as well as interstitial oedema. (H& Ex 200)

Fig. 5: Testis of diabetic rats treated with 5 % DP showing congestion of interstitial blood vessels (small arrow) and slight degeneration of spermatogonial cells lining seminiferous tubules (large arrow). (H& Ex 400)

Fig. 6: Testis of diabetic rats treated with 5 % DP showing degeneration of spermatogonial cells lining seminiferous tubules. (H& Ex 400)

Fig. 7: Testis of diabetic rats treated with 10 % DP showing apparent normal seminiferous tubules; expect some congestion of interstitial blood vessels. (H& Ex 200)

Fig. 8: Testis of diabetic rats treated with 10 % DP showing normal seminiferous tubules. (H& Ex 200)

Fig. 9: Testis of diabetic rats treated with 15 % DP showing hyperactivation and hyperplasia of germ cells with impaction of seminiferous lumen with sperms. (H& Ex 200)

Fig. 10: Testis of diabetic rats treated with 15 % DP showing hyperplasia of germ cells and impaction of seminiferous with mature sperms. (H& Ex 200)
DISCUSSION

It is well known that oxidative stress plays an important role in the pathogenesis and development of complications of two the types of DM. Numerous epidemiological and experimental evidences have demonstrated that there is a potential relationship between testicular oxidative damage during DM and male infertility [39, 40]. Several studies have shown that antioxidant treatment improves glycemic index, reduces diabetic complications and protects from oxidative damage [41, 42]. Fruits of the date palm (*Phoenix dactylifera*) are popular plants in many countries and are a vital component of arid and semiarid regions of the world. Studies have shown that *Phoenix dactylifera* L. seeds possess high antioxidant activity due to abundance of phenolic compounds and flavonoids [14]. The flavonoids content in *Phoenix dactylifera* seeds is found to be gallic acid (0.1mg/g), Rutin (0.4 mg/g) and Quercetin (0.9 mg/g) [43]. Habib et al. [14] demonstrated that, the date seed constitutes one of the highest sources of total polyphenols, exceeding tea, grapes, flaxseed, nut seeds and even date flesh. Therefore, in this experimental work we investigated the antihyperglycemic, antioxidant, antihyperlipidemic and fertility effects of date pits in STZ- induced diabetic rats.

In the present study, the chemical composition of date pits on dry matter basis revealed high percentage of carbohydrates (58.79±0.63) and fiber (21.54±0.28) and considerable percentage of protein (5.47±0.21) and total fat (8.17±0.27). The good nutritional value of date seeds is based on their dietary fiber content, which makes them suitable for the preparation of fiber-based foods and dietary supplements [44]. Our results fall within the range of date pits values previously reported by Attalla and Harraz [45], Hamada *et al.* [46], Aldhaheri *et al.* [47] and Al-Farsi *et al.* [48]. In this study, severe loss of body weight was observed in diabetic group, the mean final body weight and BWG% were significantly different when compared to control group. Injection with STZ is associated with the characteristics loss of body weight which is due to increased muscle wasting probably due to excessive utilization of protein, indicating the marked reduction of carbohydrates available to the cells [49]. Date pits improved body weight of diabetic groups significantly in a concentration dependent manner, which indicates the prevention of muscle tissue damage due to hyperglycemic condition. Ali *et al.* [50] reported that, feeding male rats with date pits for 28 days, at levels of 7% and 14% increased the final body weight significantly.

The ameliorative effect of DP may be due to its ability to reduce hyperglycemia which may be attributed to its antioxidant properties and radical scavenging activity [17].

An evaluation of sperm characteristics is useful when investigating the underlying cause of male infertility [51]. In the present study, diabetic group showed very highly significant decrease in sperm count, percentages of sperm motility and viability with very highly significant increase in percentages of abnormal sperm when compared with the control group. The effect of diabetes on these sperm end point parameters was in consistent with other reports in both rats and humans [52-54]. In diabetic rats, average sperm count of approximately 45 million/ml was lower than normal (approximately 60 million/ml per ejaculate) [55]. Oligozoospermia could predispose diabetic males to infertility [56]. Sperm with normal forward motility are capable of swimming through the female reproductive tract and ultimately fertilized the oocyte. This characteristic is largely acquired during sperm transit and storage in the epididymis [57]. Several factors including cAMP, intracellular pH and Ca$^{2+}$ level could affect ability of the sperm to display these characteristics [58]. The percentage of sperm viability was also reduced in diabetic rats which in line with reports in both rodents [18, 57] and humans [59]. Kanter *et al.* [60] reported that in rats, diabetes could induce sperm apoptosis which resulted in reduced sperm viability. Diabetes induces oxidative-stress has been reported to cause peroxidation of sperm membrane lipid which might interfere with membrane fluidity and transport processes [61]. In accordance with this evidences, the appearance of various abnormal sperm shapes could be due to abnormal membrane or cellular and nuclear changes induced by diabetes [62]. Higher sperm count, percentages of sperm motility and viability with lower percentage of abnormal sperm were observed following DP treatment to diabetic rats. There was very highly significant amelioration as compared with diabetic untreated group. The most improvement was detected at concentration 15 % DP level which suggests that DP modulate the damaging impact of diabetic on sperm.

In the present study, DM rats showed very highly significant increase in serum glucose concentration when compared to control group. This finding is in agreement with those obtained by Nelli *et al.* [55], who found highly significant hyperglycemia in diabetic group when compared to control group. This result may be explained by Szkudelski [63], who reported that, injection of STZ selectively destroyed the pancreatic ß cells leading to
diabetes mellitus. Feeding diabetic rats diets supplemented with DDP at the three concentration levels resulted in very highly significant reduction of elevated blood glucose and this further strengthens its anti-hyperglycemic activity as reported by El-Fouhil et al. [64]. The previous studies demonstrated that oral administration of date seed aqueous extract combined with insulin to STZ induced diabetic rats decreased the blood glucose level significantly toward normal compared to those of diabetic rats treated with insulin as a single drug. El-Fouhil et al. [19] suggested that, the mechanism by which date seed extract exhibits its hypoglycemic effect on diabetic rats was by stimulation of endogenous insulin secretion through extra-islet sources. The hypoglycemic effect of DP under investigation may probably due to its high fiber content, which delay glucose absorption from intestine, increase pancreatic extraction of insulin and increased insulin sensitivity at the cellular level [65]. This effect may also be explained by Habib et al. [14], who reported that date seeds possess high antioxidant activity due to abundance of phenolic compounds and flavonoids which are known to be bioactive antidiabetic principles.

It is well known that testosterone plays a critical role in sperm production and maturation. In our experiment, serum testosterone level was markedly decreased in diabetic group when compared with the control group. The reduced level of testosterone could be attributed to the toxic effect of STZ on male fertility, through disruption of testicular functions with consequent decrease in testosterone level [66, 67]. The reduced level of testosterone may also be due to diminution of the level of insulin in STZ induced diabetic rats as evidenced by the studies of Hurtado de Catalfo et al. [68], since insulin acts as an anti-apoptotic factor capable to regulate testicular apoptosis and sexual dysfunction induced by diabetes [69]. Moreover, supplementation of DP in the diets of diabetic rats ameliorated the toxic effect of STZ and contributed to the normalization of serum testosterone level in a concentration dependent manner. Literature also documents the protective effect of date palm pit on toxicity produced by methylprednisolone on testis shown by significant increase in testosterone level in serum of male albino rats accompanied with increased spermatogenesis [70].

Chronic hyperglycemia induces carbonyl stress which in turn can lead to increased oxidation of lipids [71]. In the current study, it is found that lipid peroxidation level in the testicular tissues significantly increased, while the activity of SOD significantly decreased in diabetic group as compared with the control group. Our result was at par with the study of Xu et al. [8], who found increased level of TBARS and decreased level of SOD in the testicular tissule of STZ- induced diabetic, which was an indirect evidence of intensified free radicals production. The increased concentration of lipid peroxides may propagate oxidative damage by increasing peroxy and hydroxyl radicals [72]. Superoxide dismutase is one of antioxidant enzymes which play an important role in antioxidant defense, scavenges superoxide radical by accelerating its conversion to hydrogen peroxide (H$_2$O$_2$) [73]. The decreased level of SOD in diabetic rat's testicular tissue observed in this study may be attributed to increased utilization due to excessive oxidative stress. Treatment of diabetic rats with DP resulted in significant recovery of the TBARS and SOD towards the normal levels in a concentration dependent manner especially in the high dose group (15%), the levels of TBARS and SOD were almost equivalent to that in the control group. The ameliorated effect of DP on lipid peroxidation may be attributed to the antioxidants found in date seeds, especially phenolics and flavonoids [17]. Flavonoids are a diverse group of polyphenols (phenyl benzopyrans) which are phytochemicals with well known multidirectional biological activities [74].

In diabetes, hyperglycemia is accompanied with dyslipidemia [75]. In the present results, diabetic rats showed highly significant elevation in TC, TG, LDL-C and VLDL-C and highly significant reduction in HDL-C as compared with the control group. Meanwhile, diabetic rats treated with DP exhibited significant improvement in these parameters when compared with untreated diabetic group. The dyslipidemia observed in the untreated diabetic rats could indicate an increase in the mobilization of free fatty acids from the peripheral fat depots. This could result from the uninhibited actions of lipolytic enzyme lipase caused by insulin deficiency characteristic of the diabetic state [76]. Our results indicated that, the lipid lowering effect of DP might through its stimulation of the production of insulin which in turn inhibits lipoprotein lipase activity, or reduces lipid peroxidation, because of high antioxidant content. Flavonoids possess excellent antioxidant properties related to their abilities to interfere with the formation and propagation of free radicals and protect low density lipoproteins from oxidation [77]. Histopathological examination of diabetic rat's testicular tissue showed apparent alteration characterized by marked testicular degeneration and absence, degeneration and necrosis of spermatogonial cells (germ cells) lining seminiferous tubules. This result is in agreement with.
those reported by Kanter et al. [78] in which STZ could disrupt the seminiferous tubule structure causing considerable decrease in the spermatogenic cell series and atrophy of the tubules with varying degree of spermatogenetic arrest. Testicular tissue damage caused by STZ in rats could be responsible for the reduction and the death of germ cells [79]. In mammals, the mechanism of action that results in cell death has not been fully identified; however, it is thought to be as a result of DNA and chromosomal damage brought forth by mechanisms involving free radicals generation during STZ metabolism, resulting in cell death by apoptosis or necrosis via increasing the expression of cytochrome c and caspases 9 and 3, which in turn result in a high frequency of single and double stranded DNA [80, 81]. Therefore, free radicals production resulting in oxidative stress is a popular theory that explains the etiology and pathophysiology of the biological effects of diabetes mellitus, especially in regard to cell damage, cellular degeneration and subsequent complications [82].

Feeding diabetic rats diets supplemented with DP alleviated these testicular histopathological deficits especially at the concentration of 15% by providing protection against germ cell death and impairment in the spermatogenic process. This observation is in agreement with Saeed et al. [83], who found that administration of date palm pit powder to nicotine-treated albino mice resulted in improvement of spermatogenesis. Since oxidative stress and low androgenic hormone levels are the main causing factors of testicular dysfunction and impairment of spermatogenesis in diabetic rats [59, 84], the mechanism of alleviation of the testicular lesions seen in diabetic rats treated with DP, may include the antioxidant [85] and androgenic effects of flavonoids [18] and phenols [86] previously reported to be present in date seeds.

In conclusion, our study provide a clear evidence that DP possesses antihyperglycemic, antioxidant and antihyperlipidemic activities, as well as it plays a provital role in modulating testicular dysfunction induced by diabetes in male rats. This promisingly supports the use of DP as a food supplement or an adjunct treatment for diabetics. Moreover, further work is necessary to elucidate in detail the molecular mechanism of action mediated by DP.

REFERENCES


